## Remarks

The amendments to the claims include amendments made in response to suggestions by Examiners Robinson and Low during the March 29, 2004, interview. The amendments made to the claims in the response filed January 27, 2004, were not entered, and are also included herein.

Claims 14, 18, 21, 28, and 30 having been amended, claims 1-13, 20, 22-27, and 31 having been canceled, the pending claims are claims 14-19, 21, 28-30, and 32-34. Reconsideration and withdrawal of the rejections are respectfully requested.

### Support for Claim Amendments

The claim amendments presented herein include those amendments presented in the Response filed January 27, 2004.

The amendment of claims 14 and 18 to recite "at least one" and "each of the following," and the amendment of claim 30 to recite "each of the following" is supported by the specification at, for instance, the paragraph spanning pages 11 and 12.

The amendment of claim 18 to recite "wherein fermentation activity of the yeast cells of step (iii) is not decreased after culture in non-selective medium for greater than 40 generations" is supported by the specification at, for instance, page 20, line 23 through page 21, line 2. The remaining amendments of claim 18 are supported by the specification at, for instance, page 18, lines 27-31, and by claim 20 as originally filed.

The amendment of claims 14, 18, and 30 to recite a "yeast" autonomous replicating sequence is supported by the specification at, for instance, page 18, lines 27-31.

The amendment of claim 26 corrects a clerical error.

**Summary of Interview**

Examiners Robinson and Low are thanked for the courtesies extended to the undersigned during the interview of March 29, 2004. During the interview independent claims 14, 18, and 30 and the rejection of those claims under 35 U.S.C. 103(a) as obvious over Ho et al. in view of Lopes et al. were discussed. Possible amendments to the claims to put them into condition for allowance were discussed, including the amendment of claims 14 and 18 to recite "at least one" and "each of the following," and the amendment of claim 30 to recite "each of the following." The Examiners requested submission of either a Notice of Appeal or a Request for Continued Examination, and agreed to consider a written synopsis of the arguments made by the undersigned during the interview, and amendments to the claims.

**The New Matter Rejection**

The Advisory Action mailed February 2, 2004, stated that the carlier filed amendment introduced new matter for the recitation of "greater than 40 generations."

In a telephone conference with the Examiner on February 25, 2004, the support in the application for the recitation of "greater than 40 generations" was discussed. Specifically, the undersigned pointed out that the recitation was supported by the specification at, for instance, page 20, line 23 through page 21, line 2, where it states that "the newly-developed stable recombinant yeast, 1400(LNH-ST), can still ferment both glucose and xylose with equal efficiencies after being cultured in non-selective medium for 4, 20, and 40 generations . . . and has subsequently been cultured in non-selective medium for several hundred generations, and still retains full activity in cofermenting both glucose and xylose." Since the specification shows that the cells had subsequently been cultured in non-selective medium for several hundred generations, there is support for the recitation of "greater than 40 generations."

It is the applicants' understanding that the new matter rejection has been withdrawn.

**The 35 U.S.C. §112, Second Paragraph, Rejection**

The following remarks are identical to the remarks present in the response to the final rejection submitted January 27, 2004.

The Examiner rejected claims 22-28 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The cancellation of claim 22, 23, and 25 renders the present rejection moot with respect to those claims and the claims dependent thereon. In claim 28, the recitation of "a second section marker" is amended to recite "a second selection marker."

The Examiner is requested to reconsider and withdraw the rejection of claim 28 under 35 U.S.C. §112, second paragraph.

**35 U.S.C. §103 Rejection over Ho et al. and Lopes et al.**

The Office maintained the rejection of claims 1-34 under 35 U.S.C. 103(a) as obvious over Ho et al. (WO 95/13362) in view of Lopes et al. (*Yeast* 1996;12(5):467-77). Applicants respectfully maintain the traversal of this rejection. In addition to the remarks made by the applicant in the prior responses, the Examiner is requested to consider the following comments.

A. The cited documents do not teach or suggest all the claim limitations when combined

Independent method claims 14, 18, and 30 each recite a replicative and integrative plasmid comprising a "yeast autonomous replicating sequence." Independent product (vector) claims 28, 29, 34 each recite a plasmid vector comprising a functional "yeast autonomous replicating sequence." The limitation "yeast autonomous replicating sequence" is not taught or suggested by the cited art.

It is well known in the art that there are several distinct types of chimeric plasmid vectors, including "(i) YIp (yeast integrating plasmids), which are unable to replicate and transform by integration into the genome of the recipient strain; (ii) YEp (yeast episomal plasmids), which carry the replication origin of the yeast 2-μm circle, an endogenous yeast plasmid, and can replicate in the recipient cell; and (iii) YRp (yeast replicating plasmids), which can replicate utilizing yeast autonomous replicating sequences (ARS)" (see Gietz et al., BioTechniques, 30, 816-831 (2001), at page 817, col. 2 [1], submitted with the response filed January 6, 2003). Thus, the 2-μm origin of replication and the yeast autonomous replicating sequence origin of replication are recognized in the art as different replication origins.

Ho et al. disclose the use of plasmids containing the 2-μm origin of replication. For instance, at page 15, line 29 through page 16, line 31, Ho et al. disclose that plasmids disclosed therein contain the yeast 2-μm replicon. Ho et al. do not teach or suggest the use of a plasmid with a yeast autonomous replicating sequence as an origin of replication. The combination of Ho et al. with Lopes et al. would result in a plasmid containing the yeast 2-μm replicon, and not a plasmid with a yeast autonomous replicating sequence.

Two types of plasmid vectors are disclosed by Lopes et al. The pMIRY2-based plasmid vectors include yeast rDNA, a chloroplast DNA marker, the LEU2d gene, and pBR322 sequences (see Lopes et al. at page 468, column 1). The pMIRY1-based plasmid vectors include yeast rDNA, a synthetic oligonucleotide, the LEU2d gene, and pUC9 sequences (see Lopes et al. at page 468, column 2). The plasmid vectors of Lopes et al. do not include a yeast autonomous replicating sequence, nor do Lopes et al. suggest the use of a yeast autonomous replicating sequence. The combination of Lopes et al. with Ho et al would result in a plasmid containing the yeast 2-μm replicon, and not a plasmid with a yeast autonomous replicating sequence.

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[1] While Gietz et al. was published after the filing date of the present application, the quoted passage references a document published in 1979.

Since neither Ho et al. nor Lopes et al. teach or suggest a yeast autonomous replicating sequence or the use of plasmids containing a yeast autonomous replicating sequence, combining Ho et al. and Lopes et al. could not result in a plasmid having a yeast autonomous replicating sequence. Accordingly, when combined the cited documents do not teach or suggest all the claim limitations for independent claims 14, 18, 28, 29, 30, and 34.

To establish a *prima facie* case of obviousness of the claimed invention, the Office must show that (i) the prior art documents must teach or suggest all the claim limitations, (ii) motivation to modify or combine documents; and (iii) there is a reasonable expectation of success. Since the cited documents do not teach or suggest all the claim limitations for independent claims 14, 18, 28, 29, 30, and 34, the Office has not presented a *prima facie* case of obviousness. Thus, the independent claims 14, 18, 28, 29, 30, and 34 cannot be considered as obvious in view of the cited art.

B. There is no motivation to combine or modify the cited documents

Two statements are made in the Final Office Action (dated October 2, 2003) regarding the motivation to combine or modify the cited documents. Each of these statements are considered in the following two sections:

1. The Final Office Action states "[o]ne of ordinary skill in the art would be motivated to combine the teaching of both references because the method taught by Ho et al. introduces DNA into the same yeast taught by Lopes et al." (Final Office Action, page 8, first full paragraph).

The Manual of Patent Examining Procedure provides the burden the Office has in making a *prima facie* case of obviousness.

> "The initial burden is on the examiner to provide some suggestion of the desirability of doing what the inventor has done. To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." MPEP §706.02(j) (emphasis added). Moreover, "[t]he mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination." In re Mills, 16 USPQ2d 1430 (Fed Cir. 1990); MPEP §2143.01 (emphasis added).

The statement made by the Office at page 8 of the Final Office Action may show that the two documents could be combined; however, it does not provide any convincing line of reasoning as to why a skilled person would combine the documents. There is no suggestion in the cited art of the desirability of the combination.

2. The Final Office Action states it "would have been obvious to . . . arrive at the claimed invention as a whole by combining the teachings of Ho et al. and Lopes et al. because Ho et al. teach that the simultaneous fermentation of xylose and glucose into ethanol from the yeast Saccharomyces cerevisiae, as ethanol is said to be an ideal fuel for automobiles and Lopes teach a method of making transformants stable maintained in non-selective medium for multiple generations over long periods of time" (Final Office Action, page 8, first full paragraph).

To begin with, this statement does not provide any convincing line of reasoning as to why a skilled person would combine the documents. There is no suggestion in the cited art of the desirability of the combination.

Furthermore, the statement that "Lopes teach a method of making transformants stable maintained in non-selective medium for multiple generations over long periods of time" is not necessarily true.

Lopes et al. state that "[i]n this paper we describe studies aimed at establishing whether pMIRY-type plasmids can indeed be stably maintained under the conditions applied during industrial production of proteins" (page 467, col. 2), and Lopes et al. go on to clearly prove that the pMIRY-type plasmids are *not* stably maintained under certain conditions. Lopes et al. disclose that "plasmid size is a crucial factor in determining mitotic stability of the pMIRY-type vectors" (page 473, col. 2); and "the mitotic stability of pMIRY2 vectors carrying such a [foreign] gene is decreased significantly" and yeast transformed with such a vector "lose some 80% of their vector copies over a period of 70 generations of growth in non-selective medium containing galactose as the sole carbon source" (page 472, col. 2).

Moreover, the Office is requested to consider with particularity that Lopes et al. state that "[s]table maintenance is only observed when the complete plasmid has a size no larger than that of the rDNA unit (9.1 kb)" (abstract, also see page 473, col. 2, for a similar statement). This is notable because adding the DNA fragment disclosed by Ho et al., i.e., the fragment containing the genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, to the smallest plasmid taught by Lopes et al. would result in a plasmid with a size greater than 9.1 kb. The reasoning showing how such a plasmid would be greater than 9.1 kb is presented in the following paragraph.

The smallest pMIRY-type vector disclosed by Lopes et al. is pMIRY1, which has a size of about 6.1 kb. The DNA fragment disclosed by Ho et al. that includes the genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase is at least 4.9 kb in size.[2] The addition of the Ho et al. DNA fragment to the smallest pMIRY-type vector would result in a vector of at least 11 kb. This is greater than the size limit of 9.1 kb disclosed by Lopes et al., and

[2]Ho et al. disclose that the size of the translated region of the xylulokinase gene is 2.1 kb (see Example 3). Ho et al. also disclose at Example 2 that the size of the xylitol dehydrogenase and its associated promoter are 1.9 kb and 910 bp, respectively. This is a total of 4.9 kb, and does not include the xylose reductase gene.

thus will result in a vector having reduced mitotic stability.

The cited Lopes et al. document also states at page 467, column 2, that the pMIRY2 vector integrated into the yeast genome140 copies and was stably maintained in non-selective medium for multiple generations over long periods of time, and references a different document (and this different document is also authored by Lopes). However, the Lopes et al. document cited in the present rejection clearly refutes that statement. The pMIRY2 vector is the subject of the experiments described in the Lopes et al. document cited in the present rejection, and the Lopes et al. document cited in the present rejection clearly states that plasmid size is a crucial factor in determining mitotic stability of the pMIRY-type vectors.

Thus, the statement that "Lopes teach a method of making transformants stable maintained in non-selective medium for multiple generations over long periods of time" is not true when the insert includes the genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase (independent claims 18 and 29 and dependent claim 17).

C. There is no reasonable expectation of success

Applicants submit that since the combined documents do not teach or suggest all the claim limitations when combined, there is no basis for the skilled person to have a reasonable expectation of success in practicing the claimed invention. Moreover, with respect to independent claims 18 and 29, each refer to yeast having an insert that includes the genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, and wherein fermentation activity of the yeast is not decreased after culture in non-selective medium for greater than 40 generations. As discussed above, the addition of the Ho et al. DNA fragment containing genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase to the smallest pMIRY-type vector would result in a vector of at least 11 kb. This is greater than the size limit of 9.1 kb disclosed by Lopes et al., and consequently the skilled person would expect the vector to have

reduced mitotic stability.

For at least the reasons discussed above, it is respectfully submitted that the pending claims are not obvious in view of the cited art. The Office is requested to reconsider and withdraw the rejection of claims 1-34 under 35 U.S.C. 103(a) as obvious over Ho et al. (WO 95/13362) in view of Lopes et al. (*Yeast* 1996;12(5):467-77).

**35 U.S.C. §103 Rejection over Ho et al. and Hallborn et al.**

The Office maintained the rejection of claims 1-13, 23-29, 31 and 34 under 35 U.S.C. §103(a) as being unpatentable over Ho et al. (WO95/13362) in view of Hallborn et al. (Canadian Patent No. 2,090,122). Applicants note with appreciation the withdrawal of this rejection with respect to claims 14-22, 30, and 32-33, and respectfully maintain the traversal of this rejection with respect to the remaining claims 28, 29, and 34. In addition to the remarks made by the applicant in the prior responses, the Examiner is requested to consider the following comments.

A. The cited documents do not teach or suggest all the claim limitations when combined

Independent product (vector) claims 28, 29, 34 each recite a plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA, where the exogenous DNA is to be integrated into the chromosomal DNA of a target cell. The cited documents do not teach or suggest a plasmid with a yeast autonomous replicating sequence and exogenous DNA, where the exogenous DNA is to be integrated into the chromosomal DNA of a target cell.

Ho et al. disclose the use of plasmids containing the 2-μm origin of replication. For instance, at page 15, line 29 through page 16, line 31, Ho et al. disclose that plasmids disclosed therein contain the yeast 2-μm replicon. Ho et al. do not teach or suggest the use of a plasmid with a yeast autonomous replicating sequence as an origin of replication and exogenous DNA, where the exogenous DNA is to be integrated into the chromosomal DNA of a target cell. The

combination of Ho et al. with Hallborn et al. would result in a plasmid containing the yeast 2-μm replicon, and not a plasmid with a yeast autonomous replicating sequence and the exogenous DNA to be integrated into the chromosomal DNA of a target cell.

Hallborn et al. teach the use of an autonomously replicating plasmid, however, the document is silent regarding whether the replicon is a 2-μm or a yeast autonomous replicating sequence. Moreover, the autonomously replicating plasmid of Hallborn et al. *does not include the exogenous DNA that is to be integrated.* As taught by Hallborn et al., integration of exogenous DNA into the yeast genome includes introducing a DNA fragment into a yeast that is then integrated. An autonomously replicating plasmid is also introduced at the same time as the fragment, but the autonomously replicating plasmid *does not include the exogenous DNA to be integrated* into the yeast chromosome and the plasmid does not integrate; it is included because it carries a suitable marker to permit identification of transformants, and is later removed from the cells (see Hallborn et al. at page 7, lines 27-31, and page 17, lines 24-30). The combination of Hallborn et al. with Ho et al would result in a plasmid containing the yeast 2-μm replicon, and not a plasmid with a yeast autonomous replicating sequence and the exogenous DNA that is to be integrated into the chromosomal DNA of a target cell.

Thus, none of the cited documents teach or suggest a plasmid vector containing a functional yeast autonomous replicating sequence *and an exogenous DNA for use in integrating the exogenous DNA sequence* into chromosomal DNA of a target yeast cell (claims 28, 29, and 34). Since neither Ho et al. nor Hallborn et al. teach or suggest a plasmid with a yeast autonomous replicating sequence including the exogenous DNA to be integrated, combining Ho et al. and Hallborn et al. could not result in a plasmid having a yeast autonomous replicating sequence and the exogenous DNA to be integrated. Accordingly, when combined the cited documents do not teach or suggest all the claim limitations for product (vector) claims 28, 29, 34.

To establish a *prima facie* case of obviousness of the claimed invention, the Office must show that (i) the prior art documents must teach or suggest all the claim limitations, (ii) motivation to modify or combine documents; and (iii) there is a reasonable expectation of success. Since the cited documents do not teach or suggest all the claim limitations for independent claims 28, 29, and 34, the Office has not presented a *prima facie* case of obviousness. Thus, the independent claims 28, 29, and 34 cannot be considered as obvious in view of the cited art.

B. There is no motivation to combine or modify the cited documents

Two statements are made in the Final Office Action (dated October 2, 2003) regrading the motivation to combine or modify the cited documents. Each of these statements are considered in the following two sections:

1. The Final Office Action states "it would have been obvious to one of ordinary skill in the art to arrive at the claimed invention as a whole because Ho et al. and Hallborn et al. teach the fermentation of sugars to ethanol (i.e., xylose to ethanol) using the same strain of yeast" (Final Office Action, page 5, last paragraph).

The Manual of Patent Examining Procedure provides the burden the Office has in making a *prima facie* case of obvious.

> "The initial burden is on the examiner to provide some suggestion of the desirability of doing what the inventor has done. To support the conclusion that the claimed invention is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed invention or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." MPEP §706.02(j) (emphasis added). Moreover, "[t]he mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination." In re Mills, 16 USPQ2d 1430 (Fed Cir. 1990); MPEP §2143.01 (emphasis added).

The statement made by the Office at page 5 of the Final Office Action may show that the two documents could be combined; however, it does not provide any convincing line of reasoning as to why a skilled person would combine the documents. There is no suggestion in the cited art of the desirability of the combination.

2. The Final Office Action states "one of skill would have been motivated to combine the teachings of the references because Ho et al. disclose that ethanol is an ideal liquid fuel for automobiles and Hallborn et al. disclose a method to perform stable transformation over time" (Final Office Action, paragraph bridging pages 5 and 6).

There is no basis in Hallborn et al. for the statement that "Hallborn et al. disclose a method to perform stable transformation over time." Hallborn et al. does not appear to make any statements regarding the stability of the linear DNA fragment used to transform yeast. Moreover, the Office has not provided any reasoning as to why such a linear DNA fragment would be expected to be stable. Since Hallborn et al. is silent regarding the stability of the transformations, the asserted stability cannot be used by the Office to argue that the skilled worker would be motivated to combine or modify the documents.

C. There is no reasonable expectation of success

Applicants submit that since the combined documents do not teach or suggest all the claim limitations when combined, there is no basis for the skilled person to have a reasonable expectation of success in practicing the claimed invention.

For at least the reasons discussed above, it is respectfully submitted that the pending claims are not obvious in view of the cited art. The Office is requested to reconsider and withdraw the rejection of claims 28, 29, and 34 under 35 U.S.C. §103(a) as being unpatentable over Ho et al. (WO95/13362) in view of Hallborn et al. (Canadian Patent No. 2,090,122).

## Summary

It is respectfully submitted that the pending claims are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted for
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CERTIFICATE UNDER 37 CFR §1.8:
The undersigned hereby certifies that the Transmittal Letter and the paper(s), as described hereinabove, are being transmitted by facsimile in accordance with 37 CFR §1.6(d) to the Patent and Trademark Office, addressed to Assistant Commissioner for Patents, MAIL STOP AF, P.O. Box 1450, Alexandria, VA 22313-1450, on this 2 day of April, 2004, at 11:25am (Central Time).

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